

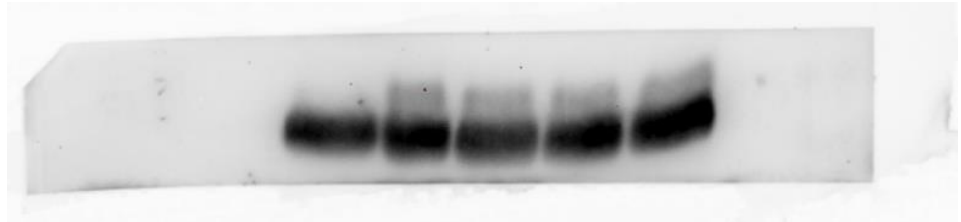
Gel 1

MM T47D MCF7 EXOS. MPC8 MPC12 MPC22 MPC8.2 MM

TSG101



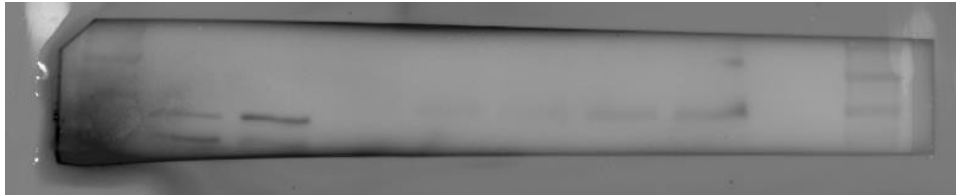
CD81



Gel 2

MM T47D MCF7 EXOS. MPC8 MPC12 MPC22 MPC8.2 MM

Calnexin



β -actin



Uncropped images of the PVDF membrane fragments used for the western blot analysis shown in Figure S1B. Due to the limited amount of protein obtained from plasma-derived exosomes, two SDS-PAGE gel were run in parallel and transferred to PVDF membranes, which were subsequently cut into sections corresponding to the molecular weight ranges of the target proteins prior to incubation with primary antibodies. Each membrane fragment was then probed independently with antibodies against the exosomal markers CD81 and TSG101 (Gel 1), and the negative control Calnexin and loading control β -actin (Gel 2).

This approach allowed the detection of multiple markers while preserving the limited sample material available. The western blot was performed for qualitative characterization of exosome preparations (presence or absence of canonical exosomal markers and absence of the endoplasmic reticulum protein Calnexin), and no quantitative comparisons were performed.

The uncropped images include two additional lanes that are not displayed in Figure S1B: (i) a commercially available lyophilized exosome preparation used as a technical positive control during assay optimization, which did not produce consistent signal for all markers under the experimental conditions, and (ii) an exosome preparation derived from a patient sample collected after disease progression. The latter was not included in the final figure as the present study focuses exclusively on plasma samples obtained at treatment initiation.

The images shown here represent the original uncropped membrane sections as acquired during the experiment.

MM: Molecular marker; EXOS.: lyophilized exosomes.